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Declaration under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
9 December 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NELL-1 ENHANCED BONE MINERALIZATION

(57) Abstract: This invention pertains to the discovery that the human NELL-1 gene induces or upregulates bone mineralization. The HELL-1 gene or gene product thus provides a convenient target for screening for modulators of bone mineralization. In addition, HELL-1 can be used to facilitate repair of bone fractures and/or to generally increase bone density.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/29281

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 15/63
US CL : 435/455
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/455

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN/WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database WPIDS on STN, No 2001-300143, TING, K. 'Screening for an agent that alters bone mineralization, useful for facilitating bone calcification or repair, comprises contacting a cell containing NELL_1 geen with a test agent and detecting change in NELL-1 gene expression level,' abstract, WO 2001024821.	1-4
X	Database CAPLUS on STN, NO. 2000:15500, KIM D. et al, NELL-1 enhances mineralization in fetal calvarial osteoblastic cells, abstract, Surgical Forum, 1999.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/29281

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1,3,6 and 7

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US03/29281

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1,3,6, and 7, partially, drawn to methods of increasing the expression or activity of Nell-1 by transfecting a cell with an exogenous nucleic acid expressing Nell-1.

Group 2, claim(s) 1,3,6, and 7, partially, drawn to methods of increasing the activity of Nell-1 by delivering to a cell an exogenous Nell-1 protein.

Group 3, claim(s) 1,2,4, and 5, partially, drawn to methods of decreasing Nell-1 expression or activity by use of anti-Nell-1 antisense, ribozyme, or RNAi.

Group 4, claim(s) 1,2,4, and 5, partially, drawn to methods of decreasing Nell-1 expression or activity by use of Nell-1-specific catalytic DNA.

Group 5, claim(s) 1,2,4, and 5, partially, drawn to methods of decreasing Nell-1 expression or activity by use of anti-Nell-1 intrabodies.

Group 6, claim(s) 1,2,4, and 5, partially, drawn to methods of decreasing Nell-1 expression or activity by knocking out Nell-1 in target cells.

Group 7, claims 23-25, partially, drawn to methods of increasing Nell-1 expression by increasing expression or activity of Msx2.

Group 8, claims 23-25, partially, drawn to methods of increasing Nell-1 expression by increasing expression or activity of Cbfa1

Group 9, claims 23-25, partially, drawn to methods of decreasing Nell-1 expression by decreasing expression or activity of Msx2.

Group 101, claims 23-25, partially, drawn to methods of decreasing Nell-1 expression by decreasing expression or activity of Cbfa1

Group 112, claims 8-22 and 26-40, drawn to methods of screening for modulators of Nell-1 expression.

Group 12, claim 41, partially, drawn to a pharmaceutical composition comprising a nucleic acid encoding a Nell-1 protein.

Group 13, claim 41, partially, drawn to a pharmaceutical composition comprising a Nell-1 protein.

Group 14, claim 41, partially, drawn to a pharmaceutical composition comprising an agent that altersa expression or activity of a Nell-1 protein.

The inventions listed as Groups 1-14 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Claim 1 is drawn to a method of modulating calvarial osteoblast differentiation and mineralization comprising altering expression or activity of Nell-1, wherein increased expression or activity of Nell-1 increases osteoblast differentiation or mineralization. However, Kim et al (Surgical Forum (1999) 50: 599-601 taught that causing overexpression of Nell-1 in either primary rat calvarial cells or MC3T3 cells resulted in an increase in mineralization. See abstract. Thus claim 1 is anticipated by the prior art, and the technical feature linking the inventions cannot be a special technical feature under PCT Rule 13.2. Also note that the expression vector of Kin also anticipates the first and third embodiments of claim 41, i.e. inventions 12 and 14. Further note that Zhang et al (Surgical Forum (2001) 52: 576-578) taught that Nell-1 overexpression in transgenic mice caused craniosynostosis in the calvarium. As such, the inventions have been restricted as shown above, and the special technical feature of each invention is deemed to be as stated above in the description of the various inventions.